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A micropuncture study of renal sodium retention in nephrotic syndrome in rats: Evidence for increased resistance to tubular fluid flow

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A micropuncture study of renal sodium retention in nephrotic syndrome in rats: Evidence for increased resistance to tubular fluid flow. Micropuncture studies were carried out in surface nephrons of rats with nephrotoxic-serum (NTS) -induced nephrotic syndrome during a period of active sodium and water retention. It was found that hydrostatic pressure and tubular diameter were increased in the proximal tubules (13.4 ± 0.2 vs. 10.4 ± 0.2 mm Hg; 31.3 ± 0.9 vs. 18.4 ± 0.7 μ), whereas pressure and tubular diameter were normal in the distal tubules. Single nephron glomerular filtration rate (SNGFR) was decreased and fractional reabsorption of fluid was markedly increased in the proximal tubules (74.1 vs. 61.7%). The increased pressure gradient between the proximal and distal tubules suggests a condition of increased resistance to flow between the proximal and distal tubules. Microinfusion of proximal tubules with an isotonic "equilibrium" solution led to little or no rise in intratubular pressure in normal rats but it led to a significant rise in nephrotic rats. When proximal tubules of normal rats were infused with a solution containing 100 mg/100 ml albumin, pressure rose to levels observed in nephrotic rats. The mechanism of the increased resistance to flow appeared to be related, therefore, to the presence of protein in the tubular fluid. Sodium retention in the nephrotic animals might be attributed to the reduction in GFR. In other types of renal disease in animals and man with comparable or greater reductions in GFR, sodium retention does not occur, however, and fractional excretion of sodium in the urine is increased in proportion to the reduction in GFR. Thus, the rise in proximal fractional reabsorption secondary to impaired fluid flow could be an important factor in the sodium retention of this disease.

Etude par microponction de la rétention rénale de sodium au cours du syndrome néphrotique chez le rat: Preuve d'une augmentation de la résistance à l'écoulement dans le tube. Des microponctions de néphrons superficiels ont été réalisées chez des rats atteints de syndrome néphrotique, déterminé par le sérum néphrotoxique, au cours de la période de rétention d'eau et de sodium. Il a été constaté que la pression hydrostatique et le diamétre tubulaire sont augmentés dans les tubes proximaux (13,4 ± 0,2 vs. 10,4 ± 0,2 mm Hg ; 31,3 ± 0,9 vs. 18,4 ± 0,7 μ), alors que la pression dans les tubes distaux, ainsi que leur diamétre, sont normaux. Le débit de filtration individuel des néphrons (SNGFR) est diminué et la réabsorption fractionnelle d'eau est augmentée dans les tubes proximaux (74,1 vs. 61,7%). L'augmentation de gradient de pression entre les tubes proximaux et distaux suggère qu'il existe une augmentation de la résistance à l'écoulement entre ces deux segments. La microperfusion des tubes proximaux avec une solution isotonique "équilibrée" ne détermine pas, ou peu, d'augmentation de la pression intratubulaire chez les rats normaux mais, chez les rats atteints de syndrome néphrotique, l'augmentation est significative. Quand les tubes proximaux des rats normaux sont perfusés avec une solution contenant 100 mg/100 ml d'albumine, la pression atteint les valeurs observées chez les rats néphrotiques. Le mécanisme de l'augmentation de la résistance à l'écoulement paraît donc être en relation avec la présence de protéines dans le liquide tubulaire. La rétention de sodium chez les animaux néphrotiques peut être attribuée à la diminution du débit de filtration glomérulaire. Cependant, au cours d'autres maladies rénales de l'homme ou de l'animal, comportant une diminution comparable ou plus grande du débit de filtration, la rétention de sodium ne survient pas et l'excrétion fractionnelle de sodium est augmentée en proportion de la réduction du débit de filtration glomérulaire. Ainsi l'augmentation de la réabsorption fractionnelle proximale secondaire à la gêne à l'écoulement pourrait-elle être un facteur important de la rétention de sodium au cours de cette maladie.

The mechanism of sodium and water retention in the nephrotic syndrome has not been explained adequately. It generally is thought that reduced plasma volume due to hypoalbuminemia leads to neurogenic and/or hormonal stimuli which acts on the kidney to cause retention of sodium and water [1]. Although a number of micropuncture studies have been reported in animals with nephrotoxic-serum-induced nephrotic syndrome [2–5], no unique information was gained as to the mechanism of sodium retention. Fractional reabsorption of sodium by the proximal tubules was found to be normal in almost all previous studies, and the site of sodium retention was presumed to be in the distal portions of the

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nephron. In most of these studies, however, the micropuncture data were obtained at variable stages of the disease process, and not specifically during the period of active accumulation of sodium and water.

The present experiments were carried out in rats in an early stage of nephrotoxic-serum-induced nephrotic syndrome, at a time when the animals were retaining sodium and water. We found that hydrostatic pressure and internal diameter were increased in the proximal tubules, but not in the distal tubules. Fractional reabsorption of fluid in the proximal tubule was increased markedly. Microinfusion of proximal tubules of nephrotic rats with an isotonic "equilibrium" solution led to a significant rise in intraluminal hydrostatic pressure, whereas infusion of tubules of normal rats caused little or no increase in pressure. Infusion of proximal tubules of normal rats with the same solution containing 100 mg/100 ml albumin led to a rise in pressure comparable to that observed in the nephrotic rats perfused with nonprotein-containing solution. The findings are consistent with the view that there is increased resistance to flow of fluid between the proximal and distal convoluted tubules in the nephrotic rats, apparently related to the presence of protein in the tubular fluid. We propose that the resulting slow flow in the proximal tubules plays an important role in the salt and water retention of the nephrotic syndrome.

Methods

Nephrotoxic serum (NTS) nephritis, which results in a nephrotic syndrome, was induced in rats using rat antisera prepared by and provided to us by Dr. R. M. Gilbert. The antibody to rat kidney was produced in rabbits as follows: kidneys from ten rats were excised, washed in isotonic saline buffered at pH 7.3 with phosphate (PBS), the capsule was stripped away, and the cortex was separated surgically from the medulla. The cortex was cut into small cubes and pushed through a #150-mesh brass sieve. The sieved cortical material was added to 100 ml of PBS, mixed, and centrifuged at 3000 rpm for 20 min. The supernatant was discarded and the tissue resuspended in 15 ml of 0.1 N Tris buffer in isotonic saline. An equal volume of complete Freund's adjuvant (Difco Laboratories, Detroit, Michigan) was added. Rabbits weighing approximately 2 kg were immunized as follows: 0.5 ml of the suspension was injected into each footpad. Two weeks later, 0.25 ml of the suspension was injected subcutaneously in four different sites. The subcutaneous injections were repeated at biweekly intervals. Blood samples were collected periodically from an ear vein, and the serum was tested for nephrotoxic potency by injecting 0.5 ml daily into the tail vein of rats for 5 consecutive days. When the antisera produced heavy proteinuria in the rats, usually after two to three biweekly subcutaneous booster injections in the rabbits, the rabbits were exsanguinated, and the sera separated and frozen. In a series of preliminary experiments, it was found that daily injection of 0.5 ml of the antisera into the tail vein of rats for 5 days, followed by 3 to 5 days, resulted in heavy proteinuria and positive sodium balance in every animal tested. If a longer period were allowed to elapse after the last injection (7 to 10 days), obvious ascites developed, and GFR was found to be less than 50% of normal.

Nephrotic rats. White, male Sprague-Dawley rats were fed a sodium-deficient diet (ICN Nutritional Biochemicals Div., International Chemical & Nuclear Corp., Cleveland, Ohio) in individual metabolic cages and were given 0.9 mEq of sodium chloride per day in 50 ml of drinking water. After 3 to 5 days on this regimen, to allow for adjustment to the diet, two or three consecutive 24-hr urine collections were obtained for measurement of sodium, water, and protein excretion. Antisera (0.5 ml) was then injected daily via the tail vein for 5 days. Two days after the last injection, one to three 24-hr urine collections were obtained again for measurement of protein, sodium, and water excretion. In some nephrotic rats used for collection of efferent arteriolar blood (group II) or for microperfusion of tubules (group III) (see below), complete balances were not obtained, but 24-hr protein excretion was measured, and the animal was used if the value was > 250 mg. When the urinary measurements had been completed, micropuncture experiments were carried out.

Group I. The methods of anesthesia, surgical preparation of the cervical blood vessels, and isolation of the left kidney for micropuncture have been described in detail previously [6, 7]. An initial arterial blood sample was obtained from the carotid artery for measurement of serum proteins, cholesterol, electrolytes, and hematocrit. Ringer's lactate solution was infused i.v. at 2 ml/hr throughout the experiment. [Methoxy-^{3}H]-inulin was administered i.v. (New England Nuclear, Boston, Massachusetts) to measure GFR and tubular fluid reabsorption. FD & C green no. 3 dye (Keystone Aniline and Chemical Co., Chicago, Illinois) was pulse-injected via a jugular vein for measurement of proximal

tubular transit time and for identification of end proximal tubular convolutions and distal tubules. Blood pressure was monitored continuously via a carotid artery [6, 7], and arterial pH, P_{CO_2}, and P_{O_2} were measured periodically throughout each experiment by a Radiometer micro blood gas analyzer. Tubular fluid was collected from the last visible proximal convolution on the surface of the kidney and from the earliest convolution of the distal tubules identified by dye appearance. Initially, tubular fluid collections were made in several rats by previously described techniques [6, 7]. It became apparent, however, that proximal tubular pressure was elevated and the tubules were dilated (see below), so that in subsequent experiments proximal tubular fluid collections were made during simultaneous monitoring of intratubular pressure with a second pipet. The technique was as follows: a pipet filled with 0.5% FD & C green dye-colored isotonic saline, attached to a water manometer, was inserted into a proximal lumen and free-flow pressure measured [8]. Prior to insertion of the micropipet, the pressure in the manometer was raised to approximately 8 to 9 mm Hg, and a stopcock connecting the manometer to the pipet was then closed. As soon as the pipet was inserted into the lumen, the stopcock was opened, and the manometer adjusted so that fluid neither entered nor left the pipet tip. Care was taken to avoid introducing fluid from the pipet into the tubular lumen. The tubing between the pipet and manometer was branched with a Y connection, leading to a Statham strain guage (model P 23 Dc), which recorded the pressure on a Grass polygraph (model 5D). A second pipet filled with Sudan black-colored castor oil was next inserted distal to the first pipet, an oil column injected, and tubular fluid collected at a rate which did not alter the previously determined free-flow hydrostatic pressure. The results of proximal tubular collections without simultaneous pressure monitoring will be presented separately in Table 6. Distal tubular collections were made in the usual way, without simultaneous monitoring of pressure. In this group of rats, tubular fluid/plasma inulin ratios (TF/P_{In}) and single nephron glomerular filtration rate (SNGFR) were calculated from proximal and distal tubular fluid collections [6, 7]. Also, the reabsorptive rate per unit of tubular volume ($C/\pi r^2$) was calculated for individual nephrons from the expression [9]:

$$C/\pi r^2 = \frac{2.3 \log (TF/P_{In})}{T} \qquad (1)$$

where T is the transit time to the collection site.

In addition to tubular fluid collections and hydrostatic pressure measurements, the internal diameters of proximal and distal tubules were measured under ×220 magnification with a finely divided eyepiece micrometer. Brush borders were included in the determination of the internal diameter, as the base of the brush border offers a clear landmark. Urine was collected from the exposed urinary bladder, as described previously [6, 7]. Six nephrotic rats were studied by this protocol.

In six additional nephrotic rats, prepared in exactly the same manner described above, samples of blood were collected from efferent arterioles on the surface of the kidney by a previously described method [7], and the hematocrit of the efferent arteriolar blood was measured [7]. Single nephron filtration fraction (SNFF) was calculated from the expression:

$$SNFF = 1 - [Hct_a (1 - Hct_e)/Hct_e(1 - Hct_a)] \times 100 \qquad (2)$$

where Hct_a is peripheral blood hematocrit and Hct_e is the efferent arteriolar blood hematocrit. Plasma flow entering individual glomeruli (SNPF) was calculated from the expression:

$$SNPF = SNGFR/SNFF \qquad (3)$$

Efferent protein concentration was calculated from arterial protein concentration, using the following:

$$Prot_e = Prot_a/1 - SNFF \qquad (4)$$

The oncotic pressure of arterial plasma (π_a) was calculated from total serum protein concentration (C) by the empirical equation $\pi_a = 2.24\ C + 0.180\ C^2$ [10]. This equation applies when the albumin/globulin concentration (A/G) ratio is < 0.4 [10], as it was in the nephrotic rats. Oncotic pressure of efferent arteriolar plasma (π_e) was calculated from efferent protein concentration, using the same empirical equation. The relatively small loss of protein from the glomerular capillaries [4] was ignored.

Group II. In four nephrotic rats, prepared by the same injection schedule as the preceding group, and excreting comparable amounts of protein, intratubular pressure was measured in the proximal tubule during microinfusion at a point distal to the pressure-measuring pipet, by using the method described by Tanner and Steinhausen [11]. The microinfusion system was as described previously [12]. The pump (Sage, model 255-2) was calibrated in vitro at three different ranges to deliver 12 to 18, 32 to 48, or 60 to 75 nl/min, respectively. The composi-

tion of the perfusion solution per 100 ml was as follows: 68 mg of monobasic potassium phosphate, 75 mg of monobasic sodium phosphate, 156 mg of dibasic sodium phosphate, 475 mg of sodium chloride, 1472 mg of mannitol (osmolality, 276 mOsm; pH, 6.72). Little or no net fluid absorption occurs in normal tubules when perfused with this isotonic "equilibrium" solution [11]. The pressure proximal to the infusion pipet was measured by the same technique described for the free-flow collections. In most instances, each tubule was infused at one rate only. After two to three tubules in a rat had been infused at the slowest rate, the infusion rate was increased, and a second set of tubules was infused. The pump was calibrated at the beginning and end of each experiment, and data accepted only if the expected rates were found, ± 2 nl/min.

Normal rats. Normal male Sprague-Dawley rats were fed the same sodium-deficient diet as the nephrotic rats, and were given 0.9 mEq/day sodium chloride in their drinking water. One or two 24-hr urine collections were obtained to measure sodium, water, and protein excretion. Three groups of animals were studied, following the same protocols described above for the nephrotic rats, with the following additional information: *Group I*. In seven rats, proximal tubular fluid collections were made during simultaneous monitoring of intratubular hydrostatic pressure, as in the nephrotic rats. Distal tubular fluid was collected without pressure monitoring. Tubular diameters were measured as in the nephrotic rats. In six additional normal rats, collections of efferent arteriolar blood were made for hematocrit measurements, and calculations of SNFF, efferent arteriolar protein concentration, and SNPF were made using Equations 2 to 4. In these normal rats, oncotic pressure of arterial and efferent arteriolar blood was calculated from the empirical equation $\pi = 1.63\ C + 0.294\ C^2$ [10]. *Group II*. Microinfusion of proximal tubules with the "equilibrium" solution was carried out in five normal rats, using the same techniques described above for the nephrotic animals. In an additional four normal rats, 100 mg/100 ml bovine albumin was added to the "equilibrium" solution, and proximal tubules were infused at the three different rates. This concentration of albumin is close to the highest values of total protein measured in the proximal tubules of nephrotic rats by Von Baeyer et al [4]. Twenty-four-hour protein excretion in their nephrotic rats was in the same range observed in our animals. Pressure proximal to the infusion pipet was measured as described above.

Analytic methods. [Methoxy-^{3}H]-inulin in plasma and tubular fluid was determined by liquid scintillation counting, as previously described [6, 7]. Plasma inulin was corrected for plasma water, determined in each animal by refractometry. Sodium and potassium in serum and urine were measured by flame photometry. Serum proteins were measured by the method of Reinhold [13], and urinary protein by the method of Tsuchiya [14]. Serum cholesterol was measured on a Technicon autoanalyzer by the single-channel method. Serum protein electrophoresis was carried out on a Beckman microzone electrophoresis apparatus. Sections were taken from the kidneys of all nephrotic animals for examintion by light microscopy, immunofluorescent staining, and electron microscopy.

All balance, renal clearance, and micropuncture data were analyzed statistically by calculating average values for each animal, and using the average to represent all individual measurements for that animal. Mean ± SEM for each group was calculated from the averages of all animals, and Student's *t* test used to compare normal controls to nephrotics. In the case of the microinfusion experiments (group II), individual measurements were used to calculate mean ± SEM.

Results

Twenty-four-hour protein excretion was measured before administration of nephrotoxic sera and 3 to 5 days after a 5-day course of injections. Protein excretion was also measured in some of the control rats. The results are shown in Table 1. In normal rats, 24-hr protein excretion was 27 mg, and, in the nephrotic animals 1 to 2 days before micropuncture study, it was 518 mg. Recovery of ingested sodium (0.9 mEq/day) was 95% in normal rats and was 74% in the nephrotic animals. Although it is uncertain whether all of the unrecov-

Table 1. Urinary protein excretion and sodium balance in normal and nephrotic rats before micropuncture study[a]

	Urine protein *mg/24 hr*	Sodium recovered in urine *% intake*	V/H_2O intake *%*
Control (N = 21)	27.1 ± 1.8	95.5 ± 1.6	89.8 ± 1.0
Nephrotic (N = 15)	517.8 ± 134.9	73.9 ± 4.3	84.1 ± 2.2
P	<0.0005	<0.0005	<0.01

[a] Sodium intake was 0.924 mEq/day. H_2O intake was 50 ml/day. V is urine output; N is number of animals. Values are means ± SEM.

ered sodium was retained by the animals, because stool sodium was not measured, the large difference in urinary recovery between the normal and nephrotic animals is consistent with positive sodium balance in the experimental rats. Recovery of ingested water was 90% in the normal rats, and 84% in the nephrotic rats ($P < 0.01$). The observations indicate that retention of sodium and water was taking place in the nephrotic animals just prior to micropuncture study.

Plasma chemistry values for the initial blood specimen at the beginning of micropuncture study are shown in Table 2. Total protein and albumin concentrations were significantly lower in the nephrotic animals, and cholesterol was markedly elevated. Serum sodium and potassium concentrations were normal in both groups, as were the hematocrit values. These data demonstrate the abnormal albumin and cholesterol levels which are expressions of the nephrotic syndrome in the experimental animals.

Table 3 presents renal clearance data obtained during micropuncture experiments. GFR in the nephrotic rats was reduced by 39%. Absolute sodium excretion rate was 47% lower in the nephrotic rats, but FE_{Na} was not statistically different in the two groups. Urine flow rate (V) was also significantly lower in the nephrotic rats than in the controls. Blood pressure was not different in the two groups.

Appearance of nephrotic kidneys. At the time of micropuncture, a small accumulation of fluid was found in the abdomen of most of the nephrotic rats. The kidneys appeared swollen and slightly pale. Under the microscope, the surface tubules looked uniformly patent with very few exceptions. When FD & C dye was pulse-injected intravenously, the dye was seen to flow through the vast majority of proximal tubules, clear entirely from the surface of the kidney, and then reappear in surface distal tubules, albeit at a slower than normal velocity (see below).

Micropuncture data (Table 4 and Figs. 1 to 4). Figure 1 shows measurements of free-flow hydrostatic pressure in proximal and distal tubules of the two groups of rats. We found that pressure in the proximal tubule of the nephrotic rats was significantly increased to 13.4 ± 0.2 vs. 10.4 ± (SEM) 0.2 mm Hg in the normal rats ($P < 0.001$). There was little or no overlap between the two groups. Hydrostatic pressure in the distal convoluted tubules was 4.9 ± 0.2 mm Hg in the normal rats and 4.5 ± (SEM) 0.2 mm Hg in the nephrotic rats (P = NS).

Figure 2 shows measurements of the internal diameters of proximal and distal tubules. The mean diameter of the proximal tubules in normal rats was 18.4 ± (SEM) 0.7 μ, and in the nephrotic rats, it was 31.3 ± (SEM) 0.9μ ($P < 0.001$). The diameter of the distal tubules was 13.4 ± (SEM) 0.6μ in the normal rats and 12.8 ± (SEM) 0.5μ in the nephrotic rats (P = NS).

Table 2. Plasma composition in normal and nephrotic rats[a]

	Total protein *g/dl*	A/G	Albumin *g/dl*	Cholesterol *mg/dl*	Na *mEq/liter*	K *mEq/liter*	Hct *%*
Control	5.41 ± 0.12	0.85 ± 0.03	2.47 ± 0.08	49.1 ± 2.5	147.5 ± 1.5	4.7 ± 0.2	47.6 ± 1.0
N	(20)	(20)	(20)	(16)	(13)	(13)	(13)
Nephrotic	4.45 ± 0.14	0.39 ± 0.04	1.21 ± 0.12	188.2 ± 20.1	148.9 ± 2.2	4.6 ± 0.1	46.4 ± 1.1
N	(19)	(19)	(19)	(19)	(13)	(13)	(13)
P	<0.0005	<0.0005	<0.0005	<0.0005	NS	NS	NS

[a] Data are for initial blood collection at beginning of micropuncture study. *N* is the number of animals; A/G, arterial plasma albumin/globulin protein ratio. Values are means ± SEM.

Table 3. Renal clearance data in normal and nephrotic rats during micropuncture studies[a]

	U/P_{In}	V *μl/min/kg*	GFR *ml/min/kg*	$U_{Na}V$ *μEq/min/kg*	U_KV *μEq/min/kg*	FE_{Na} *%*	FE_K *%*	BP *mm Hg*
Control	337.9 ± 40.8	29.9 ± 3.3	8.64 ± 0.50	2.13 ± 0.35	2.05 ± 0.43	0.17 ± 0.03	5.60 ± 1.28	119.2 ± 1.4
N	(13)	(13)	(13)	(13)	(13)	(13)	(13)	(13)
N^1	(18)	(18)	(18)	(18)	(18)	(18)	(18)	(34)
Nephrotic	303.4 ± 35.5	20.7 ± 3.0	5.26 ± 0.58	1.13 ± 0.21	1.21 ± 0.18	0.16 ± 0.04	5.81 ± 1.23	118.8 ± 2.2
N	(13)	(13)	(13)	(13)	(13)	(13)	(13)	(13)
N^1	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(39)
P	NS	<0.025	<0.0005	<0.0125	<0.05	NS	NS	NS

[a] *N* is number of animals; N^1, number of observations. Values are means ± SEM.

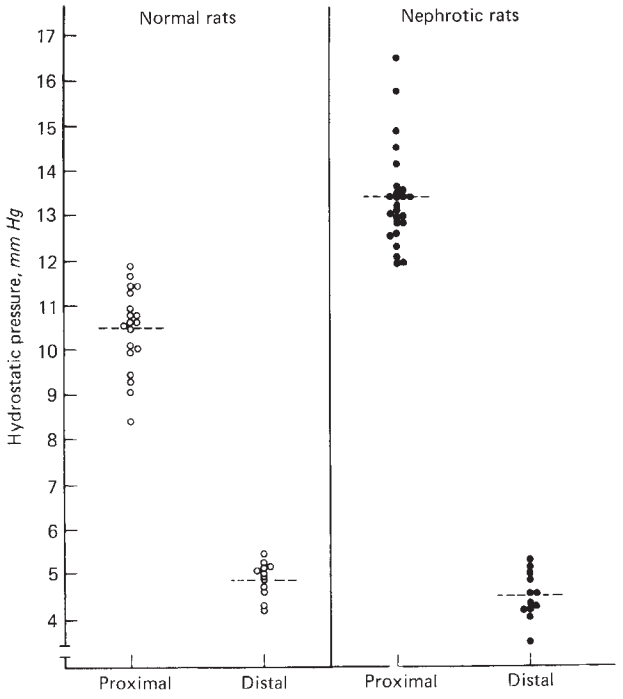

Fig 1. *Intratubular hydrostatic pressures in individual proximal and distal tubules of normal and nephrotic rats.*

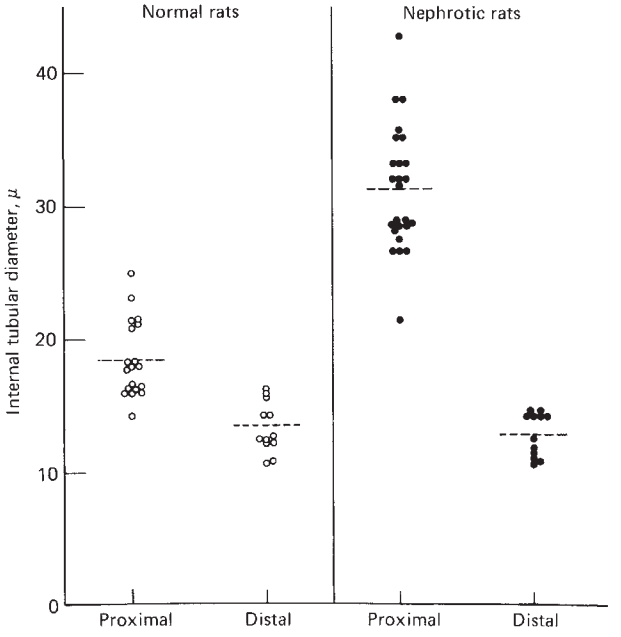

Fig 2. *Internal diameters of individual proximal and distal tubules of normal and nephrotic rats.*

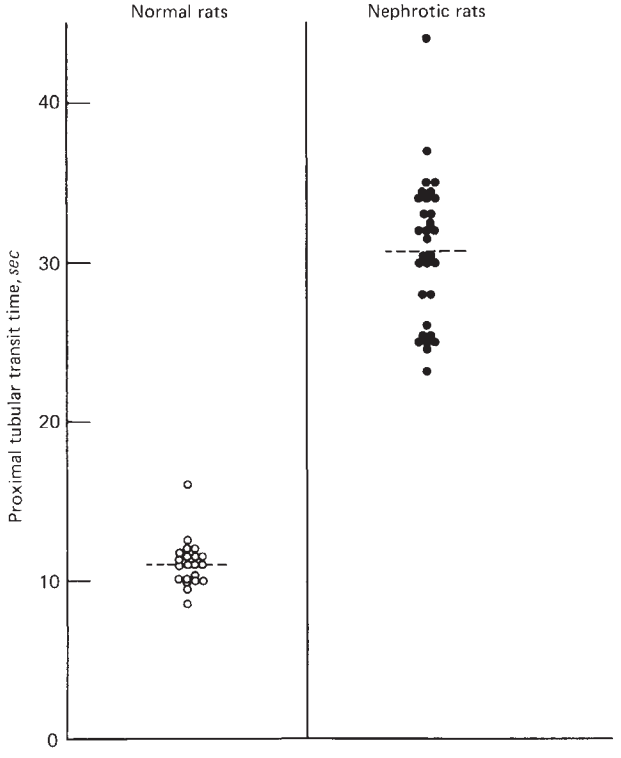

Fig 3. *Transit time of i.v. pulse-injected dye through proximal tubules of normal and nephrotic rats.*

Transit time of pulse-injected dye through the visible portions of the proximal tubule is shown in Fig. 3. The mean transit time in the normal rats was 11.2 ± (SEM) 0.5 sec, and in the nephrotic rats, it was 30.8 ± (SEM) 1.5 sec ($P < 0.0005$). There was no overlap between the two groups. Transit time to the distal tubule could not be measured accurately in the nephrotic rats because the dye appeared at widely varying times in different distal tubules. Distal transit time, however, was markedly prolonged in general in these animals.

Figure 4 shows the percent fluid reabsorption at the end of surface proximal convolutions, and the earliest distal convolutions for the normal and nephrotic rats. Mean percent reabsorption in the proximal tubule was 61.7 ± (SEM) 0.8% in the normal rats and 74.1 ± (SEM) 0.9% in the nephrotic rats ($P < 0.0005$). Early distal reabsorption was 86.2 ± (SEM) 0.6% in the normal controls and 89.2 ± (SEM) 1.2% in the nephrotic rats ($P < 0.025$). Thus, 24.5% of the glomerular filtrate was reabsorbed between the late proximal and early distal convolutions of the normal rats, but only 15% in the nephrotic rats.

Table 4 shows a summary of the micropuncture data from the group-I animals. SNGFR measured in the proximal tubules was 27.7 nl/min in the normal rats and 16.5 nl/min in the nephrotic rats, a 40% reduction. SNGFR measured from distal tubular collections was very close to values measured from proximal collections, suggesting that the nephrotic tubules had not become permeable to inulin. Tubular flow rate (TFR) at the end of the proximal convolutions, measured during simultaneous pressure monitoring, was reduced by 60% in the nephrotic rats. TFR in the early distal tubules was reduced by

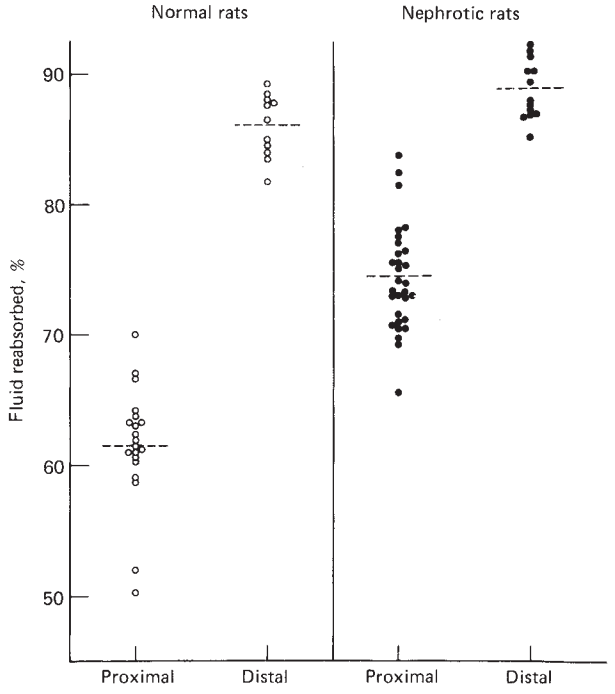

Fig 4. *Percent reabsorption of filtered water measured in late segments of surface proximal convoluted tubules and early segments of distal convoluted tubules in normal and nephrotic rats.* Points represent individual measurements.

51% in the nephrotic animals. Although fractional reabsorption was increased in the proximal tubules, absolute fluid reabsorption was 25% lower in the nephrotic rats. SNFF was not different in the two groups, but SNPF calculated from the mean SNGFR and SNFF of separate but presumably similar animals was reduced by 41%. Arterial oncotic pressure (πa) was significantly reduced in the nephrotic rats, as was oncotic pressure in the efferent arteriolar blood (πe). The values for πa and πe in the normal rats are in close agreement with data reported by others [10, 15–18]. The reabsorption rate per unit of tubular volume ($C/\pi r^2$) was reduced by 46% in the nephrotic rats, in agreement with split-droplet measurements by Rocha, Marcondes, and Malnic [5]. Absolute fluid reabsorption between the end-proximal and early distal convolutions, calculated from the mean values of TFR at each location, was considerably lower in the nephrotic vs. normal rats. Early distal TF/P_{In} ratios were significantly higher in nephrotic rats than in the normal controls.

Microinfusion experiments. The effect of infusion of proximal tubules on hydrostatic pressure measured proximal to the infusion pipet is shown in Table 5. In the normal rats microinfused at rates from 12 to 75 nl/min with an "equilibrium" solution [11], hydrostatic pressure increased only 2 mm Hg. In sharp contrast, in the nephrotic rats, hydrostatic

Table 4. Micropuncture data for normal control and nephrotic rats[a]

	TF/P_{In}	TFR *nl/min*	SNGFR *nl/min*	Absolute reab. *nl/min*	$C/\pi r^2$ *sec*$^{-1}$	SNFF %	SNPF *nl/min*	πa *mm Hg*	πe *mm Hg*
				End proximal data					
Control	2.64	10.6	27.7	17.1	0.0873	29.6	93.6	17.1	32.8
	±0.05	±0.5	±1.0	±0.6	±0.0044	±3.5		±0.7	±3.0
N	(7)	(7)	(7)	(7)	(7)	(6)	(20)	(20)	(6)
N^1	(20)	(20)	(20)	(20)	(20)	(32)		(20)	(32)
Nephrotic	4.04	4.3	16.5	12.9	0.0474	29.8	55.4	13.4	19.0
	±0.19	±0.2	±1.5	±1.1	±0.0082	±1.9		±0.6	±1.1
N	(6)	(6)	(6)	(6)	(6)	(6)		(19)	(6)
N^1	(30)	(24)	(24)	(24)	(30)	(32)		(19)	(32)
P	<0.0005	<0.0005	<0.0005	<0.005	<0.0005	NS		<0.001	<0.005
				Early distal data					
								Absolute intermediate reab. *nl/min*	
Control	7.40	3.8	27.8					6.8	
	±0.29	±0.2	±1.4						
N	(7)	(7)	(7)						
N^1	(11)	(11)	(11)						
Nephrotic	9.79	1.9	17.6					2.3	
	±1.12	±0.2	±0.9						
N	(4)	(4)	(4)						
N^1	(13)	(13)	(13)						
P	<0.025	<0.0005	<0.0005						

[a] Values are means ± SEM. Abbreviations are: TFR, tubular flow rate; $C/\pi r^2$, reabsorption rate per unit of tubular volume; SNFF, single nephron filtration fraction; SNPF, single nephron plasma flow; πa and πe, afferent and efferent arterial oncotic pressure; *N*, number of animals; N^1, number of observations.

pressure rose markedly, by as much as 9 mm Hg. In most instances, the pressure remained at a constant elevated level for 5 to 6 min of infusion. In a few cases, after an initial rise, pressure fell gradually toward the free-flow value. In normal rats microinfused with the equilibrium solution containing 100 mg/100 ml albumin, pressure rose significantly at all infusion rates, reaching values as high as in the nephrotic rats infused with a protein-free solution. These pressures remained at a constant new level as long as the infusion was continued (4 to 6 min).

Histology. Light microscopy revealed minimal hypercellularity of the glomeruli and moderate dilatation of proximal tubules in the nephrotic animals. PAS-positive material, presumably protein, was seen in the tubular lumen and loops of Henle. Immunofluorescent staining with goat antigammaglobulin showed bright linear deposits in the basement membrane of the glomeruli of all nephrotic animals, but not in the normal rats. In some instances, the glomerular deposits appeared finely granular. Deposits were seen only in glomerular tufts, and not in tubular basement membranes. Electron microscopy showed scattered subepithelial dense deposits and fusion of the food processes in the glomeruli. These findings are similar to early changes described in several other studies of nephrotoxic serum nephritis in rats [2, 3, 19, 20].

Discussion

The results of the present study suggest that in the early stage of nephrotic syndrome in rats, during active retention of sodium and water, there is increased resistance to flow of fluid between the proximal and distal tubules. We found that the hydrostatic pressure gradient between the proximal and distal tubules was significantly increased and the internal diameter of the proximal tubules was markedly widened. Because SNGFR was lower than normal and tubular fluid flow rates were very slow in the nephrotic rats, the findings of high proximal pressure and dilated tubules are most consistent with increased resistance to flow. A similar pattern of dilated tubules, reduced SNGFR, increased proximal tubular pressure, prolonged transit time, and increased fractional reabsorption of fluid is seen in obstructive nephropathy [21, 22]. In the distal convoluted tubules, hydrostatic pressure and internal diameter were the same as in normal control rats. Thus, the observations suggest that the location of the increased resistance to flow was in segments between the end of the surface proximal convolutions and the distal convoluted tubule. Increased resistance might have also been present in segments beyond the surface distal tubules but was not apparent because of the reduced delivery of fluid to this portion of the nephron. Whether this was the case or not, the functionally important site of increased resistance appeared to be between the proximal and distal convoluted tubules, since the proximal tubule manifested the significant abnormalities. Allison, Wilson, and Gottschalk [3] also found hydrostatic pressure to be increased in proximal tubules of nephrotic rats at a time when

Table 5. Intratubular pressure during microinfusion of proximal tubules of normal and nephrotic rats[a]

	Low infusion rate (12 to 18 nl/min)		Medium infusion rate (32 to 48 nl/min)		High infusion rate (60 to 74 nl/min)	
	Before *mm Hg*	During *mm Hg*	Before *mm Hg*	During *mm Hg*	Before *mm Hg*	During *mm Hg*
Normal rats (group I):						
Nonprotein equilibrium solution	10.19 ± 0.43	11.21 ± 0.22	10.91 ± 0.32	11.87 ± 0.39	10.21 ± 0.32	12.24 ± 0.77
N	(5)	(5)	(8)	(8)	(7)	(7)
P	NS		< 0.0005		< 0.005	
Nephrotic rats (group II):						
Nonprotein equilibrium solution	12.95 ± 0.30	18.17 ± 1.33	12.64 ± 0.38	18.66 ± 0.95	13.20 ± 0.75	21.98 ± 1.48
N	(9)	(9)	(10)	(10)	(7)	(7)
P	< 0.005		< 0.0005		< 0.0005	
Normal rats (group III):						
Protein-containing solution	10.62 ± 0.23	13.00 ± 0.80	11.14 ± 0.18	16.50 ± 1.32	10.52 ± 0.43	22.00 ± 1.03
N	(8)	(8)	(8)	(8)	(8)	(8)
P	< 0.0125		< 0.005		< 0.0005	
Group I vs. III	NS	NS	NS	< 0.0025	NS	< 0.0005
Group I vs. II	< 0.0005	< 0.0025	< 0.0025	< 0.0005	< 0.0125	< 0.0005

[a] Values are means ±SEM. *N* is number of tubules infused.

SNGFR was reduced. Pressures were not measured in distal tubules, and no explanation for the high proximal pressures was given [3].

The mechanism of the increased resistance to flow in the nephrotic rats is unclear. Interstitial edema of the medulla could cause compression and narrowing of the loops of Henle. Alternatively, protein precipitates (casts) in the tubular lumen might become trapped in the loop of Henle causing partial obstruction to flow. To examine these two alternative possibilities, we carried out the microinfusion experiments summarized in Table 5. Proximal tubules of normal and nephrotic rats were infused with an "equilibrium" solution that is only minimally absorbed by normal nephrons [11], and intratubular pressure was measured proximal to the infusion pipet. In normal rats, pressure rose only by 2 mm Hg at the highest infusion rates, whereas in the nephrotic rats, pressure increased by a greater amount at all rates of infusion (range, 5 to 9 mm Hg). In most instances, the pressure remained elevated as long as the infusion was continued, but in some tubules it gradually declined. When 100 mg/100 ml bovine albumin was added to the infusion fluid and proximal tubules of normal rats were infused, pressure increased significantly at all rates of infusion, and at the higher rates, pressure was comparable to that observed in the nephrotic rats. This finding suggests that protein in the tubular fluid rather than extrinsic pressure on the loop of Henle was responsible for the increase in resistance to flow. The mechanism of the acute protein effect is not clear. Whereas protein casts may have partially occluded the lumen of narrow segments of the nephron in the nephrotic rats, it seems unlikely that casts formed in the tubules of the normal rats infused for a few minutes with the protein-containing solution. Measurements of the viscosity of the protein-free and protein-containing perfusion fluids at 37° C, using a glass capillary viscometer, yielded values of 0.7048 and 0.7364 centipoise, respectively. Thus, viscosity of the protein-containing solution was only 4% higher. Although it seems unlikely that this small change can account for the findings, viscosity changes in very narrow tubes, such as the loop of Henle, might have a greater effect on resistance to flow than in large-bore tubes. Other possibilities are that the protein underwent some physicochemical change in the tubular lumen, which decreased the fluidity of tubular fluid, such as aggregation of molecules, or that an interaction occurred between the protein molecules and the brush border of the tubules. Further studies are needed to determine the nature of this effect.

The mechanism of the increase in fractional reabsorption in the nephrotic rats is uncertain, but probably cannot be accounted for by prolongation of transit time [9] or high intratubular pressure [23] *per se*. One possibility is that the greatly reduced rate of tubular fluid flow, due to a combination of reduced SNGFR and increased downstream resistance, led to increased fractional reabsorption. Bartoli and Earley [24] found in in vivo microperfusion experiments that when intraluminal flow rate is decreased, fluid reabsorption does not fall by a comparable degree, and thus fractional reabsorption increased. More recently, Andreoli, Schafer, and Troutman [25] studied the relationship between perfusion rate and tubular reabsorption in isolated rabbit proximal tubules. The absolute rate of reabsorption (Jv) was found to vary directly with perfusion rate (V^o), but the ratio Jv/V^o increased as V^o decreased. In these in vitro experiments, the bathing medium was constant, and therefore peritubular forces cannot account for the changes in fractional reabsorption with variations in luminal flow rate. Tucker and Blantz [26] studied interstitial Starling forces in rats under various experimental conditions, in order to determine controlling factors for proximal fluid reabsorption. They found that absolute proximal reabsorption correlated best with the filtered load, but not with peritubular physical forces, and concluded that glomerulotubular balance in the proximal tubule can be attributed in part to intraluminal factors. These various observations lead us to suggest that the marked slowing of proximal flow rate, due to a combination of increased downstream resistance and reduced SNGFR, was responsible for the decrease in absolute fluid reabsorption (Table 4) as well as the increase in fractional reabsorption found in the proximal tubule.

In several other micropuncture studies of NTS-induced nephrotic syndrome in rats, no significant increase in fractional reabsorption in the proximal tubules was found [2–5]. The reason for the difference is not certain, but a number of possibilities can be considered. First, sodium retention was not induced [2], or was transient [4] in two studies. In the experiments by Allison, Wilson, and Gottschalk [3], sodium retention occurred but animals were studied at widely variable periods of time after the onset of nephrotic syndrome (10 to 38 days), and marked heterogeneity of tubular function was found. If tubular function is studied at a time after ECF volume expansion has occurred, the volume expansion might restore proximal reabsorption to normal, thereby masking the abnormality which was responsible for the initial sodium retention.

Rocha, Marcondes, and Malnic [5] did find TF/P_{In} to be higher in nephrotic rats than in controls (3.23 vs. 2.53) but the difference was not statistically significant. Proximal tubular fluid collections were obtained from random points rather than terminal convolutions, and volume expansion was produced by infusion of 5% mannitol. These differences in protocol may account for the different results. In agreement with our study, Godon [27] did find increased proximal fluid reabsorption to 79% and prolonged transit time (19.2 sec) in nephrotic rats. In a study of autologous immune complex nephrotic rats, Bernard et al [28] found fluid reabsorption during volume expansion to be normal in all nephron sites up to the end of the distal convoluted tubule. Because sodium excretion was less in the nephrotic than in control rats, they concluded that either the collecting ducts or deep nephrons were the site of sodium retention. It was not shown, however, that the animals were in a stage of active sodium retention at the time of study, and volume expansion produced by infusion of saline (10% of body weight) could have obscured an abnormality in the proximal tubule.

In addition to differences in experimental protocols, we found that a technical problem exists in collecting tubular fluid from dilated tubules under increased pressure. Table 6 shows micropuncture data from two groups of nephrotic rats, one in which tubular fluid was collected without monitoring intratubular pressure and the other in which collections were made at a rate which did not alter free-flow pressure. It is clear that much higher values for SNGFR, TFR, and absolute reabsorptive rates were found when pressure was not controlled. Although TF/P_{In} was only slightly lower when pressure was not monitored, the observations in this table suggest that important artifacts may occur when fluid is collected from dilated tubules under increased pressure unless the rate of collection is controlled by pressure monitoring.

Sodium and water retention in our nephrotic rats might be attributed to the reduction in GFR, because FE_{Na} and U/P_{In} ratios in the urine were not significantly different than in the normal control rats (Table 3). It seems unlikely, however, that the decrease in GFR is the entire explanation. In rats with reductions in GFR of > 50% due to chronic pyelonephritis [29, 30] or subtotal nephrectomy [30], 24-hr sodium excretion in the awake animal is not less than in normal control animals, and overt sodium retention does not occur. FE_{Na}, measured while the animals are under anesthesia and prepared for micropuncture, is much higher than in normal controls, and $U_{Na}V$ is equal to that in controls, not reduced as in the present nephrotic animals [6, 29, 30]. In patients with nonnephrotic glomerulonephritis, decreases in GFR are thought to be responsible for an increase in total body sodium, but overt edema does not usually develop and FE_{Na} is increased in proportion to the reduction in GFR, presumably reflecting a decrease in fractional reabsorption by the tubules [1]. In sharp contrast, in some patients with a nephrotic syndrome, GFR may be normal or even elevated, and increased tubular reabsorption is clearly responsible for sodium retention [31]. Thus, reductions in GFR, unless very severe, do not seem to be responsible for edema formation [1]. In our nephrotic rats, the absence of an increase in FE_{Na} in the face of a 40% decrease in GFR implies an inappropriate tubular response, conducive to sodium retention. We postulate, therefore, that the increase in proximal fractional reabsorption found in the nephrotic rats was an important factor contributing to sodium retention. Peritubular physical forces cannot account for this increase [15], but increased resistance to flow secondary to heavy proteinuria seems to be the causative factor.

Table 6. Comparison of proximal tubular micropuncture data in nephrotic rats with and without simultaneous pressure monitoring[a]

	SNGFR *nl/min*	TFR *nl/min*	TF/P_{In}	Absolute reab. *nl/min*	GFR *ml/min/kg*	U/P_{In}
Pressure monitored	16.5 ± 1.5	4.3 ± 0.2	4.04 ± 0.19	12.9 ± 1.1	5.26 ± 0.58	303 ± 35
N	(6)	(6)	(6)	(6)	(13)	(13)
N[1]	(24)	(24)	(30)	(24)	(20)	(20)
Pressure not monitored	34.1 ± 3.4	9.1 ± 0.9	3.83 ± 0.06	25.1 ±2.4	6.39 ± 1.29	260 ± 48
N	(7)	(7)	(7)	(7)	(7)	(7)
N[1]	(26)	(26)	(26)	(26)	(14)	(14)
P	<0.0005	<0.0005	NS	<0.005	NS	NS

[a] Values are means ± SEM. *N* is the number of animals; *N*[1], the number of observations; TFR, tubular flow rate.

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References

1. GLASSOCK RJ, BENNETT CM: The glomerulopathies, in *The Kidney*, edited by BRENNER BM, RECTOR FC, Philadelphia, W.B. Saunders, 1976, pp. 950–955
2. LUBOWITZ H, MAZUMDAR DC, KAWAMURA J, CROSSON JT, WEISSER F, ROLF D, BRICKER NS: Experimental glomerulonephritis in the rat: Structural and functional observations. *Kidney Int* 5:356–364, 1974
3. ALLISON MEM, WILSON CB, GOTTSCHALK CW: Pathophysiology of experimental glomerulonephritis in rats. *J Clin Invest* 53:1402–1423, 1974
4. VON BAEYER H, VAN LIEW JB, KLASSEN J, BOYLAN JW: Filtration of protein in the anti-glomerular basement membrane nephritic rat: A micropuncture study. *Kidney Int* 10:425–437, 1976
5. ROCHA A, MARCONDES M, MALNIC G: Micropuncture study in rats with experimental glomerulonephritis. *Kidney Int* 3:14–23, 1973
6. BANK N, AYNEDJIAN HS: A micropuncture study of potassium excretion by the remnant kidney. *J Clin Invest* 52:1480–1490, 1973
7. BANK N, AYNEDJIAN HS: A micropuncture study of renal salt and water retention in chronic bile duct obstruction. *J Clin Invest* 55:994–1002, 1975
8. YARGER WE, AYNEDJIAN HS, BANK N: A micropuncture study of postobstructive diuresis in the rat. *J Clin Invest* 51:625–637, 1972
9. KOCH KM, AYNEDJIAN HS, BANK N: Effect of acute hypertension on sodium reabsorption by the proximal tubule. *J Clin Invest* 47:1696–1709, 1968
10. MADDOX DA, BENNETT CM, DEEN WM, GLASSOCK RJ, KNUTSON D, DAUGHARTY TM, BRENNER BM: Determinants of glomerular filtration in experimental glomerulonephritis in the rat. *J Clin Invest* 55:305–318, 1975
11. TANNER GA, STEINHAUSEN M: Tubular obstruction in ischemia-induced acute renal failure in the rat. *Kidney Int* 10:65–73, 1976
12. BANK N, AYNEDJIAN HS, WEINSTEIN SW: A microperfusion study of phosphate reabsorption by the rat proximal renal tubule. *J Clin Invest* 54:1040–1048, 1974
13. REINHOLD JG: Total protein, albumin and globulin, in *Standard Methods of Clinical Chemistry*, edited by REINER M, New York, Academic Press, Inc., 1953, vol. 1, pp. 88–97
14. CONSOLAZIO CF, JOHNSON RE, MAREK E: *Metabolic Methods*. St. Louis, C. V. Mosby Co., 1951, pp. 133–144
15. MADDOX DA, BENNETT CM, DEEN WM, GLASSOCK RJ, KNUTSON D, BRENNER BM: Control of proximal tubule fluid reabsorption in experimental glomerulonephritis. *J. Clin Invest* 55:1315–1325, 1975
16. BLANTZ RC, RECTOR FC JR, SELDIN DW: Effect of hyperoncotic albumin upon glomerular ultrafiltration in the rat. *Kidney Int* 6:209–221, 1974
17. BLANTZ RC: Effect of mannitol on glomerular ultrafiltration in the hydropenic rat. *J Clin Invest* 54:1135–1143, 1974
18. BLANTZ RC: Dynamics of glomerular ultrafiltration in the rat. *Fed Proc* 36:2602–2608, 1977
19. DRUET P, BARIETY J, BELLON B, LALIBERTE F: Nephrotoxic serum nephritis in the rat: Ultrastructural localization of nephrotoxic rabbit antibodies using peroxidase-labeled conjugates. *Lab Invest* 27:157–164, 1972
20. CROSSON JT, LUBOWITZ H, DEBESH C, MAZUMDAR C, WEISSER F, LANG S, ROLF D, GERMUTH FG: The production of experimental glomerulonephritis in the rat. *Arch Pathol* 98:344–350, 1974
21. RECTOR FC JR, BRUNNER FP, SELDIN DW: Mechanism of glomerulotubular balance: I. Effect of aortic constriction and elevated ureteropelvic pressure on glomerular filtration rate, fractional reabsorption, transit time and tubular size in the proximal tubule of the rat. *J Clin Invest* 45:590–602, 1966
22. WILSON DR: Micropuncture study of chronic obstructive nephropathy before and after release of obstruction. *Kidney Int* 2:119–130, 1972
23. GRANDCHAMP A, SCHERRER JR, SCHOLER D, BORNAND J: Role of luminal hydrostatic pressure in proximal tubular fluid reabsorption in the rat. *Am J Physiol* 229:813–819, 1975
24. BARTOLI E, EARLEY LE: Importance of ultrafilterable plasma factors in maintaining tubular reabsorption. *Kidney Int* 3:142–150, 1973
25. ANDREOLI TE, SCHAFER JA, TROUTMAN SL: Perfusion rate-dependence of transepithelial osmosis in isolated proximal convoluted tubules: Estimation of the hydraulic conductance. *Kidney Int* 14:263–269, 1978
26. TUCKER BJ, BLANTZ RC: Determinants of proximal tubular reabsorption as mechanisms of glomerulotubular balance. *Am J Physiol* 235:F142–F150, 1978
27. GODON JP: Evidence of increased proximal sodium and water reabsorption in experimental glomerulonephritis. *Nephron* 21:146–154, 1978
28. BERNARD DB, ALEXANDER EA, COUSER WG, LEVINSKY NG: Renal sodium retention during volume expansion in experimental nephrotic syndrome. *Kidney Int* 14:478–485, 1978
29. BANK N, AYNEDJIAN HS: Individual nephron function in experimental bilateral pyelonephritis: I. Glomerular filtration rate and proximal tubular sodium, potassium, and water reabsorption. *J Lab Clin Med* 68:713–727, 1966
30. BANK N, AYNEDJIAN HS: Individual nephron function in experimental pyelonephritis: II. Distal tubular sodium and water reabsorption and the concentrating defect. *J Lab Clin Med* 68:728–739, 1966
31. DIRKS JH, SEELY JF, LEVY M: Control of extracellular fluid volume and the pathophysiology of edema formation, in *The Kidney*, edited by BRENNER BM, RECTOR FC JR, Philadelphia, W. B. Saunders, 1976, pp. 521–522